

Supplementary materials

Table S1 - Relative abundance Phylum-level before/after RAIT and volunteers

| | Mean \pm SD | | |
|------------------------|--------------------|--------------------|--------------------|
| Phylum | Before RAIT | After RAIT | Volunteers |
| Firmicutes | 46.25 \pm 17.64% | 43.29 \pm 17.81% | 50.78 \pm 14.88% |
| Bacteroidetes | 39.26 \pm 21.06% | 42.31 \pm 19.72% | 33.31 \pm 11.78% |
| Actinobacteria | 8.66 \pm 13.27% | 4.93 \pm 5.85% | 6.20 \pm 5.38% |
| Proteobacteria | 5.22 \pm 8.25% | 5.92 \pm 10.05% | 8.62 \pm 12.31% |
| Verrucomicrobia | 2.54 \pm 6.77% | 3.15 \pm 7.21% | 0.98 \pm 1.99% |

Table S2 - Genus level- relative abundance before/after RAIT

| | | Mean | SD |
|----------------------------|--------|--------|--------|
| <i>Akkermansia</i> | before | 1.72% | 4.31% |
| | after | 3.15% | 7.21% |
| <i>Alistipes</i> | before | 10.03% | 6.40% |
| | after | 10.90% | 7.02% |
| <i>Bacteroides</i> | before | 13.25% | 12.94% |
| | after | 13.84% | 9.93% |
| <i>Phocaeicola</i> | before | 5.11% | 4.27% |
| | after | 6.48% | 5.86% |
| <i>Bifidobacterium</i> | before | 4.27% | 6.46% |
| | after | 2.79% | 4.04% |
| <i>Blautia</i> | before | 1.37% | 1.45% |
| | after | 0.93% | 1.05% |
| <i>Lachnospiraceae_u_g</i> | before | 3.34% | 3.49% |
| | after | 3.15% | 2.53% |
| <i>Firmicutes_u_g</i> | before | 4.23% | 5.22% |
| | after | 3.39% | 3.98% |
| <i>Clostridium</i> | before | 1.73% | 1.58% |
| | after | 1.74% | 2.38% |
| <i>Ruminococcaceae_u_g</i> | before | 3.38% | 2.68% |
| | after | 3.67% | 3.46% |
| <i>Dialister</i> | before | 1.08% | 2.22% |
| | after | 1.67% | 4.37% |
| <i>Dorea</i> | before | 1.30% | 2.17% |
| | after | 0.94% | 1.96% |
| <i>Escherichia</i> | before | 2.06% | 3.97% |

| | | | |
|-------------------------|--------|-------|-------|
| | after | 3.67% | 9.62% |
| <i>Faecalibacterium</i> | before | 2.78% | 3.04% |
| | after | 3.02% | 3.19% |
| <i>Gemmiger</i> | before | 2.66% | 5.39% |
| | after | 1.62% | 1.91% |
| <i>Lactobacillus</i> | before | 0.80% | 1.42% |
| | after | 0.40% | 0.91% |
| <i>Parabacteroides</i> | before | 3.96% | 3.21% |
| | after | 4.09% | 3.71% |
| <i>Prevotella</i> | before | 3.76% | 7.92% |
| | after | 3.67% | 9.33% |
| <i>Roseburia</i> | before | 2.38% | 3.34% |
| | after | 1.74% | 2.06% |
| <i>Ruminococcus</i> | before | 2.25% | 1.82% |
| | after | 1.82% | 2.48% |
| <i>Subdoligranulum</i> | before | 3.28% | 3.40% |
| | after | 4.21% | 6.69% |

Table S3 - Relative abundance genus level in volunteers

| | Mean | SD |
|---------------------|--------|-------|
| Akkermansia | 0.98% | 1.99% |
| Alistipes | 11.04% | 4.90% |
| Bacteroides | 10.00% | 6.63% |
| Phocaeicola | 4.02% | 3.19% |
| Bifidobacterium | 3.64% | 4.12% |
| Blautia | 1.27% | 1.25% |
| Lachnospiraceae_u_g | 3.70% | 2.03% |
| Firmicutes_u_g | 4.99% | 5.77% |
| Clostridium | 2.58% | 2.02% |
| Ruminococcaceae_u_g | 3.16% | 3.38% |
| Dialister | 1.93% | 2.49% |
| Dorea | 0.62% | 0.64% |
| Escherichia | 2.51% | 4.47% |
| Faecalibacterium | 7.06% | 3.02% |
| Gemmiger | 3.39% | 2.73% |
| Lactobacillus | 0.06% | 0.13% |
| Parabacteroides | 2.58% | 2.27% |
| Prevotella | 2.78% | 4.18% |
| Roseburia | 1.80% | 1.64% |
| Ruminococcus | 0.96% | 0.98% |
| Subdoligranulum | 5.03% | 5.89% |

Table S4 – Species relative abundance from controls

| | Mean | SD |
|-------------------------------------|-------|-------|
| <i>Escherichia coli</i> | 2.49% | 4.44% |
| <i>Akkermansia muciniphila</i> | 0.93% | 1.92% |
| <i>Bifidobacterium longum</i> | 1.66% | 1.86% |
| <i>Bifidobacterium adolescentis</i> | 0.50% | 0.59% |
| <i>Subdoligranulum_u_s</i> | 5.00% | 5.90% |
| <i>Ruminococcus_u_s</i> | 0.47% | 0.37% |
| <i>Ruminococcaceae_u_s</i> | 3.16% | 3.38% |
| <i>Ruminococcus bromii</i> | 0.07% | 0.22% |
| <i>Gemmiger formicilis</i> | 3.34% | 2.76% |
| <i>Firmicutes_u_s</i> | 4.99% | 5.77% |
| <i>Faecalibacterium prausnitzii</i> | 4.50% | 2.21% |
| <i>Faecalibacterium_u_s</i> | 2.55% | 1.06% |
| <i>Clostridium_u_s</i> | 2.02% | 1.68% |
| <i>Blautia_u_s</i> | 1.12% | 1.20% |
| <i>Prevotella copri</i> | 2.10% | 3.88% |
| <i>Phocaeicola vulgatus</i> | 2.34% | 2.51% |
| <i>Phocaeicola dorei</i> | 0.89% | 1.12% |
| <i>Parabacteroides_u_s</i> | 0.97% | 1.00% |
| <i>Bacteroides_u_s</i> | 3.72% | 2.50% |
| <i>Bacteroides uniformis</i> | 3.08% | 2.52% |
| <i>Alistipes putredinis</i> | 4.39% | 2.51% |
| <i>Alistipes shahii</i> | 1.35% | 1.21% |
| <i>Alistipes_u_s</i> | 1.54% | 1.31% |

Table S5 - Species-level relative abundance before/after RAIT

| | | Mean | SD |
|-------------------------------------|--------|-------|-------|
| <i>Escherichia coli</i> | before | 1.95% | 3.95% |
| | after | 3.34% | 9.55% |
| <i>Akkermansia muciniphila</i> | before | 1.62% | 4.33% |
| | after | 3.00% | 7.18% |
| <i>Bifidobacterium longum</i> | before | 0.77% | 1.13% |
| | after | 0.61% | 0.98% |
| <i>Bifidobacterium adolescentis</i> | before | 2.44% | 5.17% |
| | after | 1.16% | 1.86% |
| <i>Subdoligranulum_u_s</i> | before | 3.27% | 3.39% |
| | after | 4.21% | 6.69% |
| <i>Ruminococcus_u_s</i> | before | 1.15% | 0.87% |
| | after | 1.04% | 1.39% |
| <i>Ruminococcaceae_u_s</i> | before | 3.38% | 2.68% |
| | after | 3.67% | 3.46% |
| <i>Ruminococcus bromii</i> | before | 0.80% | 0.85% |
| | after | 0.67% | 1.15% |
| <i>Gemmiger formicilis</i> | before | 2.66% | 5.39% |
| | after | 1.61% | 1.91% |
| <i>Firmicutes_u_s</i> | before | 4.23% | 5.22% |
| | after | 3.39% | 3.98% |
| <i>Faecalibacterium prausnitzii</i> | before | 1.70% | 1.96% |
| | after | 1.76% | 1.86% |
| <i>Faecalibacterium_u_s</i> | before | 1.08% | 1.15% |
| | after | 1.25% | 1.50% |
| <i>Clostridium_u_s</i> | before | 1.40% | 1.59% |
| | after | 1.42% | 2.28% |
| <i>Blautia_u_s</i> | before | 0.83% | 0.83% |
| | after | 0.58% | 0.64% |
| <i>Prevotella copri</i> | before | 2.72% | 6.48% |
| | after | 2.25% | 6.54% |
| <i>Phocaeicola vulgatus</i> | before | 2.30% | 2.38% |
| | after | 3.39% | 3.43% |
| <i>Phocaeicola dorei</i> | before | 1.55% | 1.75% |
| | after | 1.75% | 1.75% |
| <i>Parabacteroides_u_s</i> | before | 1.87% | 1.86% |
| | after | 1.96% | 2.23% |
| <i>Bacteroides_u_s</i> | before | 5.15% | 4.84% |
| | after | 5.46% | 3.84% |
| <i>Bacteroides uniformis</i> | before | 3.87% | 4.63% |
| | after | 3.74% | 3.23% |
| <i>Alistipes putredinis</i> | before | 3.53% | 2.92% |
| | after | 3.85% | 3.44% |

| | | | |
|-------------------------|--------|-------|-------|
| <i>Alistipes shahii</i> | before | 0.99% | 1.03% |
| | after | 1.16% | 1.18% |
| <i>Alistipes_u_s</i> | before | 1.73% | 1.47% |
| | after | 1.99% | 1.64% |

Table S6 – LefSe analysis Controls versus Thyroid cancer

| Feature | Log Highest Mean | Cohort | Lda | P-Value |
|-------------------------------------|-------------------|---------|--------------------|--------------------|
| <i>Faecalibacterium prausnitzii</i> | 4.653579129618571 | Control | 4.19516870809131 | 0.000247119841581 |
| <i>Faecalibacterium_u_s</i> | 4.406887556045944 | Control | 3.913419462969283 | 0.0013830875381884 |
| <i>Clostridiales_u_s</i> | 4.220074400520429 | Control | 3.7090532118609154 | 0.0399084228452192 |
| <i>Ruminococcus_u_s</i> | 4.061622015937191 | TC | 3.507459385718969 | 0.0074205407929063 |
| <i>Ruminococcus bromii</i> | 3.905062379064919 | TC | 3.549936798276648 | 0.003091664128723 |
| <i>Bacteroides faecis</i> | 3.786637689700976 | Control | 3.282148053378805 | 0.0206167465848876 |
| <i>[Clostridium] symbiosum</i> | 3.367560952570651 | Control | 3.017963388398882 | 0.0436778750197986 |
| <i>Alistipes onderdonkii</i> | 3.919430809994788 | TC | 3.442509789012845 | 0.0289901213340677 |

Table S7 –Change in absolute abundance phylum level before/after RAIT. after the Bonferroni correction

| Phylum | Thyroid cancer patients | |
|-----------------|---|------------------------|
| | Change in absolute abundance (%) ^a | p-value ^{b,c} |
| Actinobacteria | -43.3 [-69.2 to 52.5] | 1.000 |
| Proteobacteria | 14.9 [-52.6 to 216.7] | 0.550 |
| Bacteroidetes | 5.2 [-35.5 to 88.7] | 0.960 |
| Firmicutes | -4.7 [-51.4 to 37.7] | 1.000 |
| Verrucomicrobia | -8.6 [-87.7 to 52.2] | 1.000 |

a – median [IQR]

b – Bonferroni correction

c – Wilcoxon signed ranks test

Table S8 – Change in absolute abundance genus level before/after RAIT. p-values after the Bonferroni correction

| Genus | Thyroid cancer | |
|---------------------|---|------------------------|
| | Change in absolute abundance (%) ^a | p-value ^{b,c} |
| Dorea | -28.6 [-79.7 to 69.5] | 1.000 |
| Phocaeicola | 4.2 [-46 to 99.9] | 1.000 |
| Lactobacillus | -63 [-93.8 to -6.8] | 0.105 |
| Firmicutes_u_g | -42.9 [-69.3 to 54.2] | 1.000 |
| Prevotella | 29.3 [-71.1 to 202] | 1.000 |
| Ruminococcus | -23.6 [-79.4 to 104] | 1.000 |
| Clostridium | -31.2 [-63 to 32.8] | 1.000 |
| Faecalibacterium | -19.7 [-51 to 96.5] | 1.000 |
| Ruminococcaceae_u_g | -9.6 [-48.6 to 74.7] | 1.000 |

| | | |
|---------------------|------------------------|-------|
| Akkermansia | -8.6 [-87.7 to 52.2] | 1.000 |
| Bifidobacterium | -52.6 [-81.3 to 7.7] | 1.000 |
| Blautia | -47.7 [-80.4 to 85.2] | 1.000 |
| Subdoligranulum | -30.3 [-65 to 65.9] | 1.000 |
| Gemmiger | -33.2 [-54 to 70.5] | 1.000 |
| Roseburia | -29.2 [-68 to 144.2] | 1.000 |
| Escherichia | 25.1 [-91.9 to 150.8] | 1.000 |
| Bacteroides | 16.3 [-28.5 to 76.1] | 1.000 |
| Lachnospiraceae_u_g | -25.6 [-61.3 to 110.3] | 1.000 |
| Dialister | -26.7 [-92.3 to 11.1] | 1.000 |
| Alistipes | -11.6 [-51.2 to 127.7] | 1.000 |
| Parabacteroides | -7.5 [-46.1 to 98.4] | 1.000 |

Table S9 – Change in absolute abundance species-level before/after RAIT. p-values after the Bonferroni correction

| Species | Thyroid cancer | |
|------------------------------|---|------------------------|
| | Change in absolute abundance (%) ^a | p-value ^{b,c} |
| Blautia_u_s | -48.3 [-81.9 to 38.6] | 1.000 |
| Ruminococcus_u_s | -23.7 [-70.3 to 183.1] | 1.000 |
| Ruminococcus.bromii | -67 [-97.1 to -5.4] | 0.322 |
| Firmicutes_u_s | -42.9 [-69.3 to 54.2] | 1.000 |
| Alistipes.putredinis | 2.1 [-41.8 to 133.8] | 1.000 |
| Parabacteroides_u_s | -15.7 [-62.7 to 87.6] | 1.000 |
| Bacteroides.uniformis | 1.5 [-59.7 to 68.1] | 1.000 |
| Phocaeicola.vulgatus | -8.3 [-42.5 to 108.7] | 1.000 |
| Akkermansia.muciniphila | -8.2 [-99.6 to 64] | 1.000 |
| Alistipes.shahii | 3.2 [-41.3 to 53.1] | 1.000 |
| Escherichia.coli | 41.5 [-86.4 to 297.8] | 1.000 |
| Faecalibacterium_u_s | -22.9 [-49.8 to 59] | 1.000 |
| Phocaeicola.dorei | -2.6 [-46.4 to 73.1] | 1.000 |
| Bifidobacterium.longum | -63.4 [-92.8 to -9.5] | 0.345 |
| Faecalibacterium.prausnitzii | -20.8 [-46.9 to 131.5] | 1.000 |
| Subdoligranulum_u_s | -30.3 [-64.9 to 65.7] | 1.000 |
| Alistipes_u_s | -6.5 [-44.1 to 119.4] | 1.000 |
| Clostridium_u_s | -39.9 [-76.8 to 76.9] | 1.000 |
| Ruminococcaceae_u_s | -9.6 [-48.6 to 74.7] | 1.000 |
| Prevotella.copri | -58.3 [-88.6 to 46.4] | 1.000 |
| Gemmiger.formicilis | -33.2 [-63.9 to 70.5] | 1.000 |
| Bifidobacterium.adolescentis | -66.6 [-88.7 to -18.8] | 1.000 |
| Bacteroides_u_s | -0.9 [-48.8 to 82.2] | 1.000 |

Table S10 – Change in relative abundance phylum level before/after RAIT. p-values after the Bonferroni correction

| Phylum | Thyroid cancer | |
|--------|--|------------------------|
| | Change in relative abundance (%) ^a | p-value ^{b,c} |

| | | |
|-----------------|-----------------------|-------|
| Actinobacteria | -28.2 [-67.6 to 34.5] | 1.000 |
| Proteobacteria | -0.2 [-42 to 158.2] | 0.810 |
| Bacteroidetes | 5.7 [-16.9 to 49] | 1.000 |
| Firmicutes | -10.2 [-28.8 to 23.8] | 1.000 |
| Verrucomicrobia | 2.3 [-92.3 to 94.3] | 1.000 |

a – median [IQR]

b – Bonferroni correction

c – Wilcoxon signed ranks test

Table S11 – Change in relative abundance genus-level before/after RAIT. p-values after the Bonferroni correction

| Genus | Thyroid cancer | |
|---------------------|--|------------------------|
| | Change in relative abundance (%) ^a | p-value ^{b,c} |
| Dorea | -25.7 [-76 to 94.9] | 1.000 |
| Phocaeicola | 40.2 [-34.6 to 89] | 0.966 |
| Lactobacillus | -67.9 [-93.3 to 4.3] | 0.231 |
| Firmicutes_u_g | -41.5 [-71.7 to 70.5] | 1.000 |
| Prevotella | -25.8 [-60.7 to 167.1] | 1.000 |
| Ruminococcus | -31.9 [-70.2 to 68.4] | 1.000 |
| Clostridium | -24.7 [-61.2 to 37.5] | 1.000 |
| Faecalibacterium | 4.6 [-41 to 52.7] | 1.000 |
| Ruminococcaceae_u_g | -9.1 [-36.3 to 44.6] | 1.000 |
| Akkermansia | 2.8 [-89.1 to 94.3] | 1.000 |
| Bifidobacterium | -40.3 [-80 to 29.9] | 1.000 |
| Blautia | -49 [-73.9 to 44.3] | 1.000 |
| Subdoligranulum | -14.3 [-47.2 to 58.2] | 1.000 |
| Gemmiger | -17.6 [-56.9 to 62.1] | 1.000 |
| Roseburia | -17.7 [-63.3 to 66.8] | 1.000 |
| Escherichia | -11.9 [-92.3 to 198.7] | 1.000 |
| Bacteroides | 15.4 [-28.4 to 63.7] | 1.000 |
| Lachnospiraceae_u_g | 8.4 [-61.9 to 85.3] | 1.000 |
| Dialister | -30 [-98.2 to 8.3] | 1.000 |
| Alistipes | -6.1 [-38.2 to 93.4] | 1.000 |
| Parabacteroides | -1.6 [-42.4 to 79.5] | 1.000 |

Table S12 – Change in relative abundance species-level before/after RAIT. p-value after the Bonferroni correction

| Species | Thyroid cancer | |
|----------------------|--|------------------------|
| | Change in absolute abundance (%) ^a | p-value ^{b,c} |
| Blautia_u_s | -32.3 [-76.3 to 39] | 1.000 |
| Ruminococcus_u_s | -27.3 [-70 to 66.7] | 1.000 |
| Ruminococcus.bromii | -66.3 [-92.7 to 17.1] | 0.966 |
| Firmicutes_u_s | -41.5 [-71.7 to 70.5] | 1.000 |
| Alistipes.putredinis | 1.4 [-32.5 to 108.9] | 1.000 |

| | | |
|------------------------------|------------------------|-------|
| Parabacteroides_u_s | -13.5 [-52.9 to 89.4] | 1.000 |
| Bacteroides.uniformis | -18.3 [-46.3 to 66.2] | 1.000 |
| Phocaeicola.vulgatus | 29.9 [-31.3 to 93.5] | 1.000 |
| Akkermansia.muciniphila | 3 [-99.5 to 116.1] | 1.000 |
| Alistipes.shahii | 6.6 [-16.6 to 40.6] | 1.000 |
| Escherichia.coli | -14.9 [-87.1 to 323.1] | 1.000 |
| Faecalibacterium_u_s | 4 [-32.9 to 67.3] | 1.000 |
| Phocaeicola.dorei | 21.3 [-47.6 to 53.2] | 1.000 |
| Bifidobacterium.longum | -52.7 [-86.4 to 15.9] | 1.000 |
| Faecalibacterium.prausnitzii | 7.5 [-31.9 to 75.3] | 1.000 |
| Subdoligranulum_u_s | -13.9 [-47.1 to 58] | 1.000 |
| Alistipes_u_s | 2 [-27.5 to 106.2] | 1.000 |
| Clostridium_u_s | -10.1 [-75 to 48.4] | 1.000 |
| Ruminococcaceae_u_s | -9.1 [-36.3 to 44.6] | 1.000 |
| Prevotella.copri | -49 [-88.5 to 1.6] | 1.000 |
| Gemmiger.formicilis | -17.6 [-66.2 to 62.1] | 1.000 |
| Bifidobacterium.adolescentis | -43.5 [-83.4 to 21] | 1.000 |
| Bacteroides_u_s | 12 [-33.6 to 61.9] | 1.000 |